Environmental Health Perspectives
Vol. 21, pp. 1–5, 1977

Recent Findings on the Carcinogenicity of Chlorinated Olefins

by Cesare Maltoni*

Data are presented on factors affecting the carcinogenic effects of chlorinated olefins, such as molecular structure, concentration, length of treatment, route of administration and animal species, strain, sex, and age. The observations are based upon carcinogenicity experimental bioassays of vinyl chloride and vinylidene chloride.

Early results, which appear to show that some of these factors (particularly species, strain, and sex) act by affecting the metabolism of the tested compounds, are presented, and the need for metabolic characterization of experimental animals in chemical carcinogenesis is stressed.

A project of integrated research on the carcinogenicity of different halogenated and related compounds has been going on at the Bologna Institute of Oncology and Tumour Center since 1971, and additional programs in this area are under way or under study.

Past and ongoing experiments deal with vinyl chloride (VC), vinylidene chloride (VDC), styrene, acrylonitrile, dichloroethane, chlorofluorocarbons, trichloroethylene, carbon tetrachloride, and vinylidene fluoride.

The experiments were performed by the same group in a very standard way, thus providing homogeneous and comparable information.

One is now becoming increasingly more aware that a great deal of this information may help not only to learn the effects of the particular compounds under study and their mechanism of action, but to better understand the basic factors involved in carcinogenesis in general.

Factors Affecting Neoplastic Response

The present report deals with the results obtained in our studies on the carcinogenicity of VC and VDC, pointing out the major factors affecting the neoplastic response.

The factors considered are: molecular structure, concentration, length of treatment, route of administration, animal species, strain, sex, age, and their relevance will be illustrated with VC and VDC.

Influence of the Molecular Structure

By comparing the carcinogenic activity of VC with that of VDC it can immediately be seen that molecules of very similar structure, such as vinyl chloride and vinylidene chloride, may have very different biological effects (Table 1).

While VC has been shown to affect many organs of different animal species, i.e., to be a clear multipotential carcinogen, VDC, to the present, has produced oncogenic effects definitively only in one organ (kidney) of a single species (mouse) (*1–4*).

Vinyl Chloride:
$CH_2 = CHCl$

Vinylidene Chloride:
$CH_2 = CCl_2$

Influence of Concentration

The studies on VC have already confirmed the dose-response relationship effect (*1-3*). Furthermore, they show that different concentrations, though within a narrow range of dose, may greatly affect the relative proportion of different types of tumors caused by VC (Table 2).

*Institute of Oncology and Tumour Center, Bologna, Italy.

Table 1. Effects of chemical structure: comparative oncogenic effects of VC and VDC on rodents.

Compound	Species	Tumors											
		Angiosarcomas of liver	Angiosarcomas and angiomas of other sites	Nephroblastomas	Adenocarcinomas of kidney	Sebaceous carcinomas[a]	Other cutaneous skin tumors	Tumors of lung	Tumors of brain	Hepatomas	Mammary carcinomas	Forestomach papillomas and acantomas	Lymphomas and leukemias
VC	Rat	+	+	+		+	+		+	+	+	+	
	Mouse	+	+			+	+	+			+	+	
	Hamster (Golden)	+	(+)				+					+	(+)
VDC	Rat												
	Mouse				+								
	Hamster (Chinese)												

[a]Including Zymbal gland carcinomas.

Table 2. Effects of concentration.[a]

Groups	Concentration of VC, ppm	No. of animals (Sprague-Dawley rats)	No. of animals with tumors	
			Liver angiomas	Nephroblastomas
I	200	120	12	3
II	150	120	5	7
III	100	120	1	10
IV	None	120	0	0

[a]Exposure by inhalation to VC in air, at 200, 150, 100 ppm, 4 hr/day, 5 days/week, for 52 weeks. (Results after 143 weeks = end of the experiments.)

Influence of the Length of Treatment

The schedule of treatment and especially the length of administration, may also greatly affect the neoplastic response in VC carcinogenesis (Table 3).

Influence of the Route of Administration

The route of administration of VC may significantly vary the type of neoplastic response (Table 4), probably by affecting the distribution of the compound and its metabolites in the body.

Influence of Animal Species

The animal species is a very important factor in carcinogenesis. Studies on VC carcinogenesis have shown that the range of VC-dependent tumors greatly varies from species to species, though some, such as liver angiosarcomas, are observed in all species tested (*1–3*).

Recent result with VDC (*4*) have pointed out that

Table 3. Effects of length of treatment.[a]

Groups	*Concentration* of VC, ppm	Length of *treatment*, weeks	No. of animals (*Sprague-Dawley* rats)	Animals with liver angiosarcomas	
				No.	%
I	10,000	52	60	9	15
II	6,000	52	60	13	22
III	10,000	17	60	0	—
IV	6,000	17	60	1	0.6
V	10,000	5	120	0	—
VI	6,000	5	120	0	—
VII	None	—	500	0	—

[a]Exposure by inhalation to VC in air at 10,000 and 6,000 ppm, 4 hr/day, 5 days/week, for 52, 17, and 5 weeks. (Results after 155 weeks = end of the experiments.)

Table 4. Effects of route of administration.[a]

Groups	VC treatment		No. of animals (Sprague-Dawley) rats)	No. of animals with tumors		
	Concentration	Route		Liver angiosarcomas	Nephroblastomas	Zymbal gland carcinomas
I	10,000 ppm	Inhalation	60	9	5	16
II	6,000 ppm	Inhalation	60	13	4	7
III	50 mg/kg	Ingestion	80	16	2	1
IV	16.65 mg/kg	Ingestion	80	9	3	2

[a]Exposure to VC by inhalation in air at different concentrations, 4 hr/day, 5 days/week, for 52 weeks (Group I and II), and by stomach tube in olive oil, at different concentrations, 4-5 days/week (Groups III and IV). (Results after 136 weeks = end of the experiments.)

the only type of tumor known at present as definitively VDC-dependent, the renal adenocarcinoma, is observed in mice but not in the other two tested species, i.e., rats and Chinese hamsters, though rats have been treated with higher doses (Table 5).

Influence of Strain

Differences have been observed among strain in VC carcinogenesis.

As an example, the onset of Zymbal gland carcinomas, following the treatment with this monomer, varies greatly in Sprague-Dawley and Wistar rats, though the spontaneous onset is exceptionally rare even in the most sensitive strain (Table 6).

Influence of Sex

If VDC had been tested only on female Swiss mice, probably the knowledge of its capacity to produce kidney adenocarcinomas would not have come to light (Table 7).

Influence of Age

Hepatocarcinogenesis by VC is a striking example of the influence of age in neoplastic response (Table 8).

Table 5. Effects of species.[a]

Groups	Concentration of VDC, ppm	Animals		Animals with kidney adenocarcinomas	
		Species and strain	No.	No.	%
I	200-150	Rats Sprague-Dawley	120	0	—
II	100	Rats Sprague-Dawley	60	0	—
III	50	Rats Sprague-Dawely	60	0	—
IV	25	Rats Sprague-Dawley	60	0	—
V	None	Rats Sprague-Dawley	200	0	—
VI	25	Mice Swiss	300	25	8.3
VII	None	Mice Swiss	200	0	—

[a]Exposure by inhalation to VDC in air at different concentrations, 4 hr/day, 4-5 days/week, for 52 weeks. (Results after 98 weeks = ongoing experiments.)

Table 6. Effects of strain.[a]

Groups	Concentration of VC, ppm	Animals (male rats)		Animals with Zymbal gland carcinomas	
		Strain	No.	SD	W
I	10,000	Sprague-Dawley	30	10	
II	10,000	Wistar	30		1
III	6,000	Sprague-Dawley	30	3	
IV	6,000	Wistar	30		0
V	2,500	Sprague-Dawley	30	1	
VI	2,500	Wistar	30		0
VII	500	Sprague-Dawley	30	3	
VIII	500	Wistar	30		0
IX	None	Sprague-Dawley	30	0	
X	None	Wistar	30		0
Total				17	1

[a]Exposure by inhalation to VC in air at different concentrations, 4 hr/day, 4-5 days/week, for 52 weeks. (Results after 143 weeks = end of the experiments.)

Table 7. Effects of sex.[a]

Groups	VDC treatment	Animals (Swiss mice)		Animals with kidney adenocarcinomas	
	ppm	Sex	No.	No.	%
I	25	male	150	24	16
II	25	female	150	1	0.7
III	None	male	190	0	—
IV	None	female	190	0	—

[a]Exposure by inhalation to VDC in air at 25 ppm, 4 hr/day, 4-5 days/week, for 52 weeks. (Results after 98 weeks = ongoing experiments.)

However it becomes more and more evident that experimental and biological factors affecting the neoplastic response in chemical carcinogenesis as well as other possible toxic effects may act by determining the metabolic pathway of the tested compounds.

Recent research has shown that VC and VDC do not act *per se*, but through products of metabolic transformation, probably epoxy-derivatives.

Experiments performed in our laboratories appear to indicate that species, strain and sex greatly affect the production of active metabolites, which in turn are responsible for toxic effects of VDC (Table 9) (5).

Table 8. Effects of age.[a]

Groups	VC treatment, ppm	Age	No. of animals		No. of liver tumors	
			Total	Survivors	Angiosarcomas	Hepatomas
I	10,000	13 weeks	120	16	—	1
II	6,000	13 weeks	120	15	—	—
III	None	13 weeks	249	55	—	—
IV	10,000	1 day	46	8	10	15
V	6,000	1 day	43	5	10	13

[a]Incidence of hepatic tumors (angiosarcomas and hepatomas) among Sprague-Dawley rats, exposed to VC in air at 10,000 and 6,000 ppm, 4 hr/day, 5 days/week, for 5 weeks, at ages 13 weeks or 1 day. (Results after 135 weeks = end of the experiments).

Explanation of Role of Factors

How the experimental and biological factors which have been considered and other possible ones determine the neoplastic response, is a matter of hypothesis and inference, more or less based upon experimental evidence.

To explain the influence of some biological parameters (such as species and strain), one has first to consider the genetic basis of responsiveness of different tissues and organs in various types of animals, often expressing itself in the onset of spontaneous neoplasia in the same tissue and organ.

The major regressive and necrotic changes produced by intoxication are found in liver and kidneys.

In the case of Sprague-Dawley rats and Swiss mice there is a clear cut parallelism between toxic and carcinogenic effects of VDC (in relation to species and sex).

As far as Balb/c, C3H, and C57BL mice are concerned, we are now undertaking long-term studies to assess if there is the same parallelism (in relation to strain).

Table 9. Comparative acute toxic effects of vinylidene chloride (VDC), at 200 ppm in air, 4 hr/day for 2 days.

Species	Strain	Sex	No.	No. of survivors/day									Performance	
				1	2	3	4	5	6	7	8	9	Weight[a]	status[b]
Rats	Sprague-Dawley	M	60	60	60	60	60	59	59	59	59	59	(+)	(+)
		F	60	60	60	60	60	60	60	60	60	60	+	−
Mice	Swiss	M	60	31	26	24	20	16	11	10	10	9	−	+++(+)
		F	60	60	60	60	60	60	60	60	60	60	=	−
	Balb/c	M	30	25	12	6	6	6	6	6	6	6	−	++(+)
		F	30	30	30	30	30	30	30	30	30	30	=	−
	C3H	M	30	30	25	17	17	15	14	14	14	13	−	++
		F	30	22	19	19	19	19	19	19	19	19	=	+(+)
	C57BL	M	30	28	25	23	23	23	23	23	23	23	−	+(+)
		F	30	30	30	30	30	30	30	30	30	30	−	−

[a]Code: −, decrease; =, no change; +, enhancement (on 5th day).
[b]Code: −, no effect; +, slight effect; ++, moderate effect; +++, marked effect; ++++, profound effect. Recovery has been observed on nearly all the survivors on the 5th day.

Should it be confirmed, we do believe that there will be new routes for establishing priorities for long-term carcinogenicity bioassays, for choosing the best experimental animal models and for the understanding of mechanism of action of many organic carcinogens.

REFERENCES

1. Maltoni, C., and Lefemine, G. Le potenzialità dei saggi sperimentali nella predizione dei rischi oncogeni ambientali. Un esempio: il cloruro di vinile. Atti Accad. Nazl. Lincei, Rend. [8]56: (1974).
2. Maltoni, C., and Lefemine, G. Carcinogenicity bioassays of vinyl chloride: current results. In: Toxicity of Vinyl Chloride-Polyvinyl Chloride, I. J. Selikoff and E. C. Hammonds, Eds., New York Academy of Sciences, 1975, pp. 195-218.
3. Maltoni, C., et al. La cancerogenesi ambientale e professionale: nuove prospettive alla luce della cancerogenesi da cloruro di vinile. Ospedali Vita 1 (5-6): 4 (1974).
4. Maltoni, C., et al. Carcinogenicity bioassays of vinylidene chloride. Research plan and early results. Med. Lavoro, in press.
5. Maltoni, C., Maioli, P., and Patella, V. The effect of animal species, strain and sex on acute toxicity of vinylidene chloride. Med. Lavoro in press.